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Kin selection, relatedness, and worker control of reproduction in a large-colony epiponine wasp, *Brachygastra mellifica*

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Hamilton's kin selection theory predicts conflicts of interest among relatives, even within highly cooperative social insect societies. Because workers are the most numerous caste, collective worker interests may be an important force in determining the outcome of conflicts. In this study, we used genotypes from two DNA microsatellite loci to show that two kinds of collective worker interests are satisfied in *Brachygastra mellifica,* a member of the multiqueen epiponine wasps. First, from the high relatedness of queens (0.66) and the fact that queens are singly mated (shown by genotyping their stored sperm), we calculated that new queens are reared in colonies with a harmonic mean of 1.2 old queens, whereas males are reared in colonies with much higher queen numbers. This split sex ratio result is predicted under worker control. It matches other studies of epiponines, but *B. mellifica* has much larger mature colonies (averaging 7951 adults) with many more queens (averaging 398), showing the pattern holds for large-colony species. Second, we report the first genetic data on parentage of males in epiponines and show that these are also consistent with collective worker interests. Workers are on average significantly more related to queens than to other workers ($r = .37$ versus .23) and should therefore suppress each other and allow the queens to lay haploid (male) eggs. Though many workers have developed ovaries and could lay eggs, the genetic analyses showed that most or all males come from queens. *Key words: Brachygastra mellifica,* eusociality, genetic relatedness, mate number, microsatellites, split sex ratios, Vespidae, worker policing. *[Behav Ecol 9:573–581 (1998)]*

Hamilton's (1964a,b, 1972) theory of inclusive fitness revolutionized the study of social insects in two ways. First, it offered an explanation for the paradox of how nonreproductive workers could be favored by selection; they can compensate for their loss of direct reproduction by the indirect reproduction achieved through helping relatives, provided relatedness is high enough. Evidence has since accumulated that most social insect colonies do consist of close relatives (reviewed by Crozier and Pamilo, 1996). Hamilton (1972) considered wasps of the tribe Epiponini (Carpenter 1993) to be a possible exception. These wasps, a successful group of about 200 neotropical species, are characterized by multiple queens (Hughes et al., 1993; Jeanne, 1991), which could result in very low relatedness within colonies. If this were true, explaining worker altruism would be difficult, particularly since females are not morphologically differentiated into distinct worker and queen castes (Richards, 1978). However, recent genetic studies have shown that relatedness is not very low; relatedness among workers is typically in the range of 0.27–0.49 (Gastreich et al., 1993; Queller et al., 1993a,c; Strassmann et al., 1991, 1992, 1997b; West-Eberhard, 1990).

Having offered an explanation for the cooperation of social insects, inclusive fitness theory then turned the tables, predicting that cooperation may not be as extensive as it appears. Because even sterile workers have indirect reproduction, they should evolve in ways that maximize their own inclusive fitnesses, even if this is not ideal from the standpoint of the queen or of the colony as a whole. Predictions of worker–queen conflict have been supported by empirical studies, particularly of worker–queen conflict over sex ratios (Mueller, 1991; Queller et al., 1993c; Queller and Strassmann 1998; Sundström, 1994; Sundström et al. 1996; Trivers & Hare, 1976). How reproduction is controlled and how conflicts are limited are important issues not only for social insect evolution, but also in the evolution of any cooperative entity, including those we now regard as individuals (Buss, 1987; Maynard-Smith and Szathmáry, 1995).

In this context too, the epiponine wasps are a particularly interesting group. Several features of their biology seem conducive to conflict. First, many queens compete for reproductive rights. Second, though relatedness is not as low as might have been thought, it is still well below the three-quarters maximum in haplodiploid insect societies, and so the potential for conflicts may be larger than in many other groups with higher relatedness. Finally, workers might strive for direct reproduction, either by laying male eggs or by becoming inseminated and functioning as queens.

As the most numerous caste, workers may be in a position to enforce their interests, provided they have a common collective interest (Trivers and Hare, 1976). In the epiponine wasps, collective worker control has been suggested as the ultimate cause of the surprisingly high worker relatednesses alluded to above; worker control results in a colony cycle called cyclical monogyny, where new queens are not produced until the colony has lost all but one queen. Then a large new generation of queens is produced, mates, and lays eggs in the nest. The queen cycle is not linked to the swarming cycle (Strassmann et al., 1998). Males, on the other hand, are produced by colonies with high queen numbers. This colony cycle therefore causes high queen relatedness, which in turn results in moderately high relatedness among the queens' progeny (Queller et al., 1993c).

The logic of this sequence follows from split sex ratio theory

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(Boomsma and Grafen, 1991; Pamilo, 1991). When queen numbers vary across colonies, workers should favor rearing females (new queens) in colonies with few queens and males in colonies with many queens. This prediction stems from the asymmetrical relatedness of female workers to sisters and brothers in haplodiploids. Workers in single-queen colonies can capitalize on the high three-quarters relatedness to sisters by specializing on female brood (new queens), while those in multiple-queen colonies cannot and are therefore left to specialize on males. Both genetic and observational studies confirm the prediction that daughter queens are produced in colonies with few original queens and males are produced in colonies with more original queens (Gastreich et al., 1993; Hughes et al., 1993; Forsyth, 1978; Queller et al., 1993a,c; Strassmann et al., 1991, 1992, 1997b; West-Eberhard, 1978). The highly related cohorts of new queens mate and reproduce on their natal colony and, in certain circumstances, cleave off in groups with workers in a process called swarming (Jeanne, 1991; West-Eberhard, 1982). Queens have never been seen to found nests independently or to insinuate themselves into other colonies.

Collective worker interests might also determine colony behavior in another area: who lays the eggs that become males. Because males arise from unfertilized eggs, they could be laid by uninseminated workers. It is always theoretically best for a worker to raise her own sons ($r = 1$, using regression coefficients uncorrected for reproductive value; Hamilton, 1972) compared to any other males. But if workers lay the male eggs, then workers will generally be rearing the sons of other workers, not just their own. Therefore, if workers collectively control whether the workers or queens produce males, they should choose the caste to whom they are most closely related (Ratnieks, 1988; Starr, 1984; Woyciechowski and Łomnicki, 1987). Sometimes this will result in worker policing, with workers acting against each other to permit the queens to lay eggs (Ratnieks and Visscher, 1989; Visscher, 1996). In epiponines, the relatednesses relevant to worker preferences for who should produce the males has not been studied, and little is known about who actually produces the males.

That workers might produce males in some epiponines, including species of *Brachygastra,* is supported by the common presence of "intermediates." This is a class of individuals that are queenlike in having somewhat developed ovaries, often with at least one mature egg. However, unlike queens, they are uninseminated (Gastreich et al., 1993; Naumann, 1970; Richards, 1978; Simões, 1977). They might be male-laying workers, but they could also be old queens that never were inseminated, new queens that have not yet been inseminated, or workers that are striving to become reproductive but do not actually lay eggs.

In this paper, we report data from *Brachygastra mellifica,* the Mexican honey wasp, that extends knowledge of collective worker interests in two ways. First, most of the work on epiponine split sex ratios noted above comes from species with colony sizes in the hundreds and average queen numbers less than 30, but other species, including *B. mellifica,* have much larger colonies with queen numbers in the hundreds (reviewed in Hughes et al., 1993; Jeanne, 1991). Some researchers suggest that such large-colony species, or even the largest colonies in small-colony species, may never reach a single-queen stage (Jeanne, 1991; Richards, 1978; West-Eberhard, 1978), which would result in very low relatedness among workers and the brood they rear. There are at least three reasonable grounds for this belief: (1) multiple queens could be necessary to maintain egg-laying rates in large colonies; (2) the amount of queen reduction required to achieve monogyny is much greater; (3) monogynous colonies have not been found in some species. Thus, it may be premature to claim that cyclical monogyny and split sex ratios are general in the tribe until large-colony species are studied.

Second, we test predictions derived from the theory of worker policing regarding whether workers or queens should lay male eggs. In epiponines, worker relatedness preferences for who should produce the males have not been studied, and little is known about who actually produces them. In this study of *Brachygastra mellifica,* we report the first genetic data from epiponines for both of these questions, so we can determine whether collective worker interests are satisfied with respect to male production.

Questions of relatedness and parentage can be addressed with genetic markers, and DNA microsatellites are a particularly good kind of genetic marker due to their codominant Mendelian behavior and their high variability (Queller et al., 1993b). In this study, although we used only two microsatellite loci (originally found in other polistine wasps species, *Parachartergus colobopterus,* and *Polistes bellicosus,* Strassmann et al., 1996b, 1997a), they proved adequate to answer the questions concerning cyclical monogyny, split sex ratios, worker interests, and male parentage.

MATERIALS AND METHODS

Wasp collection

We studied *Brachygastra mellifica* at the northernmost edge of its range in the lower Rio Grande Valley of southern Texas, USA (Sugden and McAllen, 1994). It is also found throughout Mexico and Central America (Richards, 1978). We collected colonies from Hidalgo, Cameron, and Willacy counties, 22–23 June 1995, 8–10 August 1995, and 1 April 1996 (Table 1). We placed newspaper advertisements to locate colonies because they are not dense, though not uncommon in the area. At no site was another active colony nearby, which makes mixing of workers among colonies unlikely. We collected six mature colonies (W2-1, W2-3, W2-4, W3-2, W3-6, W4-1), one recently founded colony (W3-1), and the remnants of a colony that had recently swarmed and was largely vacated (W3-3; swarming had been observed by the owners of the property). We also collected samples of adults from four active, mature colonies of minimum dimension 30 cm × 25 cm, which is consistent with a colony population of at least 3500 wasps. All colonies are located within a 40 × 80 km^2 area (N-S, E-W) at about 26.2° N (Table 1). A smaller 40 × 8 km^2 area included all but the two colonies from Indian Lake and the sample from Lyford (Table 1). The entire area was divided into three demes for the purposes of testing for population subdivision, with the single outlying sample from Lyford excluded from the analysis. We collected the nests from bushes or trees at heights ranging from 1 m to 4 m, avoiding higher nests because of collecting difficulties.

We collected the nests at night when all wasps were likely to be present. We trimmed away all but one or a few main support branches, enclosed the nest in a large plastic garbage bag, secured the opening, and then cut the remaining branches. Few wasps escaped collection. From the four nests that were sampled instead of collected, we used a hand-held vacuum specially rigged to suck wasps from the surface of the nest into a plastic cup attached to the bottom of the device, or netted wasps in flight immediately outside the nest. These surface samples did not include any males or queens. We froze all wasps collected on dry ice or in a −80°C freezer for later analysis.

Colony composition

We dissected a subsample of wasps from each colony to get estimates of the numbers of males, queens, and workers. For

Table 1
Characteristics of the study colonies

Nest	Nest dimensions (cm)	Nest weight (kg)	No. of wasps collected	No. dissected	No. of queens (%)	No. of males (%)	No. of workers (%)	% workers with mature eggs	Date collected	Location	Worker relatedness ±SE (*n*)
W2-1	66.0 × 21.5	3.1	18,698	903	227 (1.2)	1017 (5.4)	17,454 (93.4)	24.2	22 Jun 95	Weslaco	0.28 ± 0.05 (20)
W2-3	29.0 × 26.0	1.2	3496	271	133 (3.8)	53 (1.5)	3310 (94.7)	26.7	23 Jun 95	Weslaco	0.00 ± 0.12 (20)
W2-4	38.0 × 27.0	2.4	5563	709	376 (6.8)	0 (0.0)	5187 (93.2)	16.5	23 Jun 95	Indian Lake	0.41 ± 0.32 (20)
W3-1	13.5 × 13.5	0.2	624	145	90 (14.4)	0 (0.0)	534 (85.6)	13.1	8 Aug 95	Donna	0.18 ± 0.22 (20)
W3-2	48.0 × 28.0	1.1	4157	275	64 (1.5)	211 (5.1)	3882 (93.4)	18.6	8 Aug 95	McAllen	0.24 ± 0.03 (20)
W3-3	40.0 × 28.0	0.8	348	171	81 (23.3)	0 (0.0)	267 (76.7)	0.7	9 Aug 95	Indian Lake	0.45 ± 0.13 (10)
W3-6	40.5 × 28.0	2.2	11,405	420	1529 (13.4)	0 (0.0)	9876 (86.6)	21.1	10 Aug 95	Pharr	0.03 ± 0.03 (20)
W4-1	~30.0 × 25.0	1.1	4387	200	60 (1.4)	53 (1.2)	4274 (97.4)	6.8	1 Apr 96	Mercedes	0.15 ± 0.10 (20)
W2-2	—	—	99	99	—	—	—	52.5	22 Jun 95	Weslaco	0.40 ± 0.13 (20)
W2-5	—	—	33	33	—	—	—	33.3	25 Jun 95	Lyford	0.28 ± 0.15 (11)
W3-4	—	—	36	36	—	—	—	27.8	10 Aug 95	Harlingen	0.68 ± 0.18 (10)
W3-5	—	—	64	64	—	—	—	4.7	10 Aug 95	Edinburg	0.20 ± 0.17 (10)
Average				277	398	222	7331	20.4			0.29

Nest W3-1 was a newly founded colony, and nest W3-3 was the remnants of a colony which had recently swarmed.

each colony, adults from different comb sections had been separated into different bags before freezing, and we took samples from each bag to guard against the possibility that castes were clumped in different areas. No such clumped pattern was found, so we do not report results separately for each bag. However, to estimate total numbers of each kind of individual, we extrapolated separately for each bag and then summed these extrapolations to derive totals. We distinguished males from females by their reproductive organs, both external (claspers versus sting) and internal (testes versus ovaries). Four of the eight collected colonies had males. We verified that the other colonies lacked males entirely by visually scanning the undissected individuals. We assigned relative ages to individual wasps by examining the degree of sclerotization (darkening) of the cuticle of the apical edge of the fifth gastral sternite (Gastreich et al., 1993). The older the wasps, the more extensive the sclerotization (Gastreich et al., 1993; Strassmann et al., 1992). We used five age categories, with five being the youngest and one the oldest. This categorization is most accurate for comparisons within a caste because queens may darken at a slower rate than foragers.

Table 2
Characteristics of the microsatellite loci used in this study

Locus name and GenBank accession no.	Annealing temperature (°C)	Length (base pairs)	Allele frequencies (%)
Pbe424AAT	45	194	14
U64631		197	19
		200	9
		203	21
		206	4
		209	34
			Heterozygosity: 0.77
Paco3155TAG	50	160	4
U64651		163	<1
		166	48
		178	48
		184	<1
			Heterozygosity: 0.54

We distinguished workers from queens by the presence of sperm in the spermathecae of queens. Because many uninseminated females (workers) had mature eggs, we subdivided the general worker category into two further categories, those with at least one mature egg (intermediates), and those without any mature eggs. The classification of the intermediates as workers was considered tentative because the genotyping would provide additional information.

Besides being inseminated, queens also typically possessed dark reddish-brown abdominal pigmentation, whereas uninseminated females (with or without mature eggs) and males had alternating black and yellow bands on their abdomens. However, queens from colony W3-6 were minimally differentiated by color from the workers, perhaps because they were young and were produced in a recent requeening event, a hypothesis consistent with the large number of queens on that nest.

DNA microsatellites and relatedness

To estimate genetic relatednesses among colony members, we prepared DNA for the polymerase chain reaction (PCR) according to Strassmann et al. (1996a), using either the whole wasp, thorax and head, or thorax alone. For each nest and sample we selected 15 males and 10 each of queens, workers with mature eggs, and workers with no ovarian development. Fewer wasps were used if we did not have this number in our dissected sample. We used two polymorphic microsatellite loci (Table 2), one identified from *Polistes bellicosus* (locus Pbe424AAT in Strassmann et al., 1997a) and the other from *Parachartergus colobopterus* (locus Paco3155TAG in Strassmann et al., 1996b). When amplifying the loci using PCR, we incorporated [^{35}S]dATP into the products, which were visualized by autoradiography, after running the samples on denaturing gels with an M13 sequencing reaction as a size standard (Strassmann et al., 1996a). We also genotyped the sperm we extracted from the spermathecae of 43 inseminated females to determine mating frequency of the queens (Peters et al., 1995; Strassmann et al., 1996a; see also Evans, 1993).

We estimated genetic relatedness and *F* statistics using the Macintosh computer program Relatedness 4.2b (Goodnight and Queller, 1995; Queller and Goodnight, 1989). Each col-

Table 3
Genetic relatedness among colony members

	Relatedness ± half 95% CI (no. of individuals)		
	All colonies (n = 12)	Whole colonies (n = 8)	Whole colonies with males (n = 4)
All workers	0.29 ± 0.12 (201)	0.23 ± 0.15 (150)	0.17 ± 0.22 (80)
Intermediates	0.24 ± 0.17 (80)	0.23 ± 0.20 (70)	0.21 ± 0.17 (40)
Workers without mature eggs	0.28 ± 0.16 (120)	0.18 ± 0.18 (80)	0.11 ± 0.27 (40)
Workers without mature eggs to intermediates	0.21 ± 0.13 (90, 81)	0.20 ± 0.16 (70, 70)	0.18 ± 0.23 (40, 40)
Queens	—	0.66 ± 0.23 (81)	0.53 ± 0.46 (41)
All workers to queens	—	0.37 ± 0.19 (150, 81)	0.30 ± 0.31 (80, 41)
Intermediates to queens	—	0.38 ± 0.21 (70, 71)	0.35 ± 0.29 (40, 41)
Workers with no mature eggs to queens	—	0.33 ± 0.22 (80, 81)	0.24 ± 0.37 (40, 41)
Males	—	—	0.30 ± 0.36 (60)
Queens to males	—	—	0.55 ± 0.51 (41, 60)
All workers to males	—	—	0.32 ± 0.26 (80, 60)
Intermediates to males	—	—	0.36 ± 0.26 (40, 60)
Workers with no mature eggs to males	—	—	0.28 ± 0.28 (40, 60)

"All workers" combines both workers with mature eggs (intermediates) and those without. All relatedness values reported in this table are significantly different from zero (one-tailed t test, $p < .05$); n is number of colonies; numbers in parentheses are numbers of individuals.

ony was given equal weight in the estimates. The four colonies that were sampled at the nest surface contained only workers, and unless otherwise noted, their genetic data were not used in relatedness estimates except insofar as they affected the population allele frequencies. Standard errors and confidence intervals were obtained by jackknifing over colonies and assuming that the jackknife pseudovalues followed a t distribution, with one degree of freedom less than the number of colonies (Queller and Goodnight, 1989). For comparisons of relatedness, we conducted paired tests when possible, jackknifing the within-colony differences between the two relatedness values (Queller, 1994). To obtain standard errors for individual colony relatedness estimates, we jackknifed over the two loci.

Parentage of males

We determined male parentage by using a maximum likelihood method. Because both queens and workers were incompletely sampled, direct exclusion methods were impossible. Suppose we know the queen, worker, and male genotypes. We seek the maximum likelihood, L, of the parameter Q, the fraction of males drawn from the queen allele pool (Arévalo et al., 1998). For each male-producing colony, we calculate $L = K\Pi_{males}(Q\Pi_{\text{loci}}\, f_{qi} + (1 - Q)\Pi_{loci} f_{wi})$ for values of Q sampled densely between 0 and 1. K is a multinomial constant that never has to be calculated because it multiplies all Ls by a constant, leaving likelihood ratios unafffected. For each male allele considered in turn, f_{qi} and f_{wi} are the frequencies of that allele in the queens and in the workers. Pooling alleles allows us to represent multilocus combinations of genes that do not appear in the sampled individuals, but might appear in their unsampled relatives. Overall likelihoods for all colonies were calculated as the product of the colony-specific likelihoods for a given value of Q. This gives the most likely value of Q, assuming that it is the same for all colonies.

RESULTS

Background information

B. mellifica did have very large, highly polygynous colonies that potentially are problematic for maintaining high relatedness (Table 1). Mature colonies of *B. mellifica* contained an average of 7951 adult wasps (SE = 2237, n = 6, ranging up to >18,000), 398 queens (SE = 211), 222 males (SE = 148), and 7331 uninseminated females (SE = 2048). Queen number ranged from 60 to 1529, with a harmonic mean of 113 (the harmonic mean is the measure relevant to relatedness among progeny; Queller, 1993; Wade, 1985).

We found no evidence of genetic population subdivision in this population of *B. mellifica*. The inbreeding coefficient for all 12 colonies was not different from zero (f = 0.07 ± 0.10; 95% CI). For the 11 colonies excluding the Lyford geographical outlier, F statistics ± half 95% CIs were $F_{\text{ST}} = 0.10 \pm 0.26$; $F_{\text{IT}} = 0.10 \pm 0.14$; $F_{\text{IS}} = 0.00 \pm 0.09$. Therefore, we treated the samples as coming from a single population for analyses of genetic relatedness.

Several lines of evidence indicated that the adult males were produced by the colony from which they were collected and were not males from elsewhere that had come to seek matings. First, males were either not present (4 colonies) or present in large numbers (4 colonies with between 50 and 1000 males; Table 1), a pattern that seems inconsistent with a strategy in which males enter all colonies to seek matings. Nor was the pattern consistent with outside males entering only colonies producing new queens, because all four colonies with males had low worker relatedness (Table 1), whereas, as we document below, the requeening stage is characterized by high relatedness. Most important, males within a colony were highly related to each other and to other classes of colony mates (Table 3). They were also very young (average age score 4.3, SE = 0.04, n = 143), as expected if we were sampling only males who had not yet left the colony to seek mates.

We also needed to know whether to treat the "intermediates" (uninseminated individuals with ovarian development) as queens or workers. In coloration they resembled workers, though, as noted above, this is not a perfect indicator. They were younger than other workers without mature eggs, though not markedly so (age scores: 3.3 ± SE 0.04, n = 538 versus 3.0 ± SE 0.02, n = 2518; t test, t = 4.3, $p < .0001$). Like workers, and unlike queens, intermediates were present in the nest surface samples (Table 1). Most convincing, intermediates have a workerlike kin structure; all relevant comparisons in Table 3 show intermediates to be more similar to workers without mature eggs than to queens. For example, intragroup relatedness for intermediates resembles that for workers with no mature eggs (paired difference is 0.04, p = .59, two-tailed t test for difference from 0, n = 7 colonies;

excludes colony W3-3, which did not have a sample of workers with developed ovaries) but does not resemble relatedness among queens (paired difference 0.37, $p < .002$, $n = 7$). Similarly, with respect to their relatedness to males, intermediates more closely resemble workers with no mature eggs (paired difference 0.07, $p = .24$, two-tailed t test for difference from 0, $n = 4$) than they resemble queens (paired difference 0.19, $p < .14$, $n = 4$), though they are not significantly different from either in this case. Finally, with respect to relatedness to queens, intermediates are marginally different from workers (paired difference 0.07, $p = .05$, $n = 7$) but very different from queens (paired difference is 0.23, $p = .004$, $n = 7$). Therefore, unless otherwise noted, subsequent analyses treat intermediates as workers.

To determine how many times *B. mellifica* queens mate, we examined the genotypes of the sperm retrieved from the spermathecae of 43 queens. Because males are haploid, the sperm obtained from the spermatheca of a singly-mated queen should have only one microsatellite allele at each locus; multiple alleles indicate either multiple matings or contamination of the sperm sample with the queen's DNA.

We found no convincing evidence of multiple mating in any of the 43 queens we investigated, although it could be present in a minority of queens. Only one allele was present at each locus for 29 (67%) of the sperm samples from queen's spermathecae. Because the odds of two males having the identical genotype at both loci are only around 0.1, overall these results support single mating for these queens. Multiple alleles were present in the other 14 sperm samples. In six of these samples, all maternal alleles were present in the sperm sample, as expected if it is contaminated with maternal DNA. The other eight sperm samples were also consistent with maternal contamination, but in a more tenuous manner. In four samples, maternal alleles were present only at a homozygous locus and not at a heterozygous one, which might occur if the amount of maternal contamination were just sufficient to show the amount of DNA at the homozygous locus, but not sufficient for the halved amounts at each allele of the heterozygous locus. In the other four samples, faint amplifications of a heterozygous mother's sperm showed the smaller of her alleles and not the larger. This could still be consistent with contamination if the larger allele amplifies less well, which is often noticeable in heterozygotes with alleles of rather different sizes, perhaps because the longer product amplifies to completion less often.

Cyclical monogyny and split sex ratios

If *B. mellifica* undergoes cyclical monogyny, queen colony mates should be highly related, reflecting their history of being raised on nests with a single, or few, queens. Relatedness among queens is indeed very high (0.66 ± 0.23, 95% CI; see Table 3), confirming this prediction. Relatedness among the daughters of different queens should be one-quarter of this value, or 0.16. Relatedness among workers should be slightly higher than this because some are daughters of the same queen. Actual worker relatedness was 0.23 ± 0.15 (95% CL) on the whole colonies and 0.29 ± 0.12 (95% CL) for all 12 colonies (0.16 is included in the 95% CI of the former, but not the latter; Table 3). As in other epiponine wasps, queens are more highly related than are workers (paired difference 0.43, $p < .0001$, one-tailed t test for difference from 0, $n = 8$ colonies).

An alternative explanation for high within-colony relatedness might be that some queens dominate reproduction, and there was considerable variation in the number of mature eggs found in queen ovaries. Some queens (27%) had no mature eggs in their ovaries (but, except for 1 queen, did have nearly mature eggs), 19% had 1 mature egg, 14% had 2, 15% had 3, 10% had 4, and 15% had 5–12 mature eggs. However, this variance is not sufficient to increase relatedness much among the queens' daughters. Reproductive dominance by a subset of queens will increase the fraction of siblings (compared to cousins) among the brood by the amount $\overline{(Q * \mathrm{Var}\ (P))}$, the average over colonies of the product of queen number, Q, and the within-colony variance in p, the fraction of eggs laid by each queen (Queller, 1993). If the number of mature eggs in ovaries reflects relative reproductive contribution, we estimate this quantity to be only 0.017 for *B. mellifica* (using the appropriate correction for partly dissected colonies; Queller, 1993).

If workers control investment in sexuals, they should rear males on colonies with many queens and new queens on colonies with one or a few old queens (unless the queens are unrelated; Boomsma, 1993; Boomsma and Grafen, 1991; Pamilo, 1991; Queller et al., 1993c). Because queen production appears to be rare, and we probably did not collect colonies in the process of requeening, the prediction is best tested by using an indirect measure of queen number, relatedness among females (Queller et al., 1993c). Relatedness among queen colony mates ($r = .66$, Table 3) is a reflection of the number of old queens that were present when the current queens were produced. A comparable measure for queen number when males are produced is the relatedness among workers on male-producing colonies ($r = .17$, Table 3). These two values are significantly different (one-tailed unpaired t test, $p < .005$), supporting the view that colonies producing males have more queens than those producing new queens.

We can translate these relatednesses into effective queen numbers. Equation 1 gives the relationship between daughter relatedness (r), queen relatedness (r_q), and the number of queens (N):

$$r = \frac{3}{4N} + \frac{r_q}{4}\left[1 - \frac{1}{N}\right] \tag{1}$$

assuming that queens are singly mated and contribute equally to reproduction. For male-producing colonies, we substitute relatedness among workers on male-producing colonies for r; and relatedness among queens of those same colonies for r_q (we used the same colonies to control for sampling error) and solve for a queen number of 19.6. We should note that this estimate is not very stable; small changes in the r estimates can lead to much larger queen number estimates, and the dissections suggest that the true queen numbers are indeed much higher, with 64 being the lowest number of the four queen-producing colonies. For queen-producing colonies, queen relatedness is substituted for both r_q (mother queens) and r (daughters queens), and the resulting N is 1.2.

Who produces the males?

Under collective worker control, the class to which workers are most related should be their preferred caste for laying male eggs. If we use all workers, including intermediates, worker relatedness to queens is higher than worker relatedness to other workers (Table 3; paired difference 0.15, significantly different from zero, $p = .0004$, $n = 8$, two-tailed t test). We can also test for the preference of undeveloped workers (the most numerous class) for either queens or developed workers (intermediates). Here the difference is reduced, but still indicates a significant preference for queens (paired difference 0.07, $p = .04$, $n = 7$). Under either scenario, the hypothesized controlling class of workers would transmit more genes through males if they policed worker laying, allowing queens to lay the male eggs.

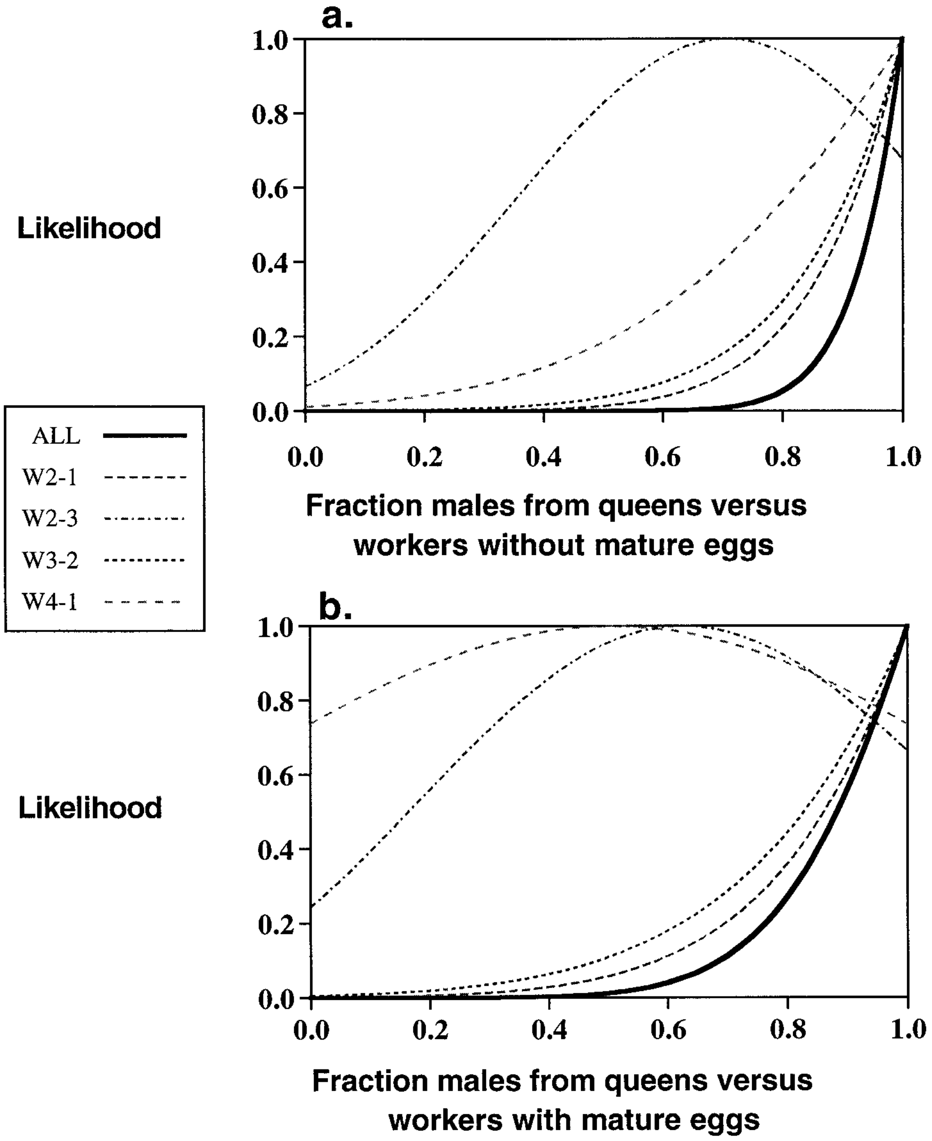

Figure 1
Maximum likelihood estimation of male production: (a) queens versus workers without mature eggs; (b) queen versus workers with mature eggs. For both, the maximum likelihood taken over all colonies (heavy lines) is that the queens produce all the males, but some colonies considered individually (lighter lines) are more ambiguous.

Queens had considerably more ovarian development than workers. Queens had an average of 2.2 mature eggs in their ovaries (SE = 0.14, range = 0–12, n = 248). Workers in the collected colonies, including intermediates, averaged only 0.23 mature eggs (SE = 0.011, range 0–6, n = 2826), that is, about a tenth the average of queens. However, because workers are more than 10 times as numerous as queens (workers average 92% of female adults; Table 1), about half of the mature eggs in ovaries resided in workers, indicating a strong potential for worker laying.

Figure 1 shows the results of the likelihood analysis for the fraction of males produced by queens as opposed to workers. The analysis is performed separately for workers with and without mature eggs. Those with mature eggs at first seem the more likely source of males, but it may be that the slightly older class of undeveloped workers is more representative (genetically) of the age class of workers that had eggs at the time these adult males were produced.

For plotting, each set of likelihoods for values of queen laying, Q, between 0 and 1 was rescaled by dividing by the maximum likelihood in that range. When undeveloped workers are used, three of the four colonies had a maximum likelihood of $Q = 1$, supporting the hypothesis that all males are produced by queens (Figure 1a). For two of these, this hypothesis was more than 20 times as likely as the alternative hypothesis that half the males are produced by workers, and for the third it is 5 times as likely. The fourth colony showed a broad likelihood curve peaking at about $q = 0.71$, suggesting the possibility of some worker laying, but complete queen laying ($q = 1$) cannot be excluded because its likelihood was not much lower. Overall, the four colonies strongly indicate that queens produce eggs that become males. When developed workers (intermediates) were used in the analysis (Figure 1b), the results were similar, except that one colony (W4-1) switched from a maximum at 100% queen production to a broad, flat curve showing that the question cannot be answered one way or the other for this colony. Overall, queen laying is clearly more likely. Though some worker laying cannot be excluded, it is not clearly supported in any colony.

The results of the likelihood analysis are reinforced by relatedness estimates (Table 2). If queens produce the males, then queen-to-male relatedness should approximate queen–queen relatedness (0.55 versus 0.53; paired t test, $p = .75$, $n = 4$) and worker-to-male relatedness should approximate worker-to-queen relatedness (0.32 versus 0.30; paired t test, $p = .75$, $n = 4$). On the other hand, if current workers produce the males, then queen-to-male relatedness should approximate queen-to-worker relatedness (0.55 versus 0.30; paired t test, $p = .05$, $n = 4$), and worker-to-male relatedness should approximate worker–worker relatedness (0.32 versus 0.17; paired t test, $p = .02$, $n = 4$). The match is good for production of males by queens and poor for production by workers.

DISCUSSION

Previous work on epiponine wasps showed that they have a colony cycle, cyclical monogyny, that is consistent with worker split sex-ratio preferences (Queller et al., 1993c). This study of *Brachygastra mellifica* extends this in two ways. First, it shows that the same pattern holds for a species with much larger colonies and a much greater degree of polygyny. Second, it shows for the first time in an epiponine wasp that who produces the males is also consistent with collective worker control.

Mature colonies of *B. mellifica* averaged almost 8000 adults, far more than the other 4 species with detailed genetic studies (*Polybia emaciata* 141; *Polybia occidentalis* 261; *Parachartergus colobopterus* 283; *Protopolybia exigua* 216; Gastreich et al., 1993; Queller et al., 1993a; Strassmann et al., 1991, 1992). Even the *B. mellifica* swarm and the colony remnant that had recently swarmed were larger than the mean colony sizes for the other species. The mean number of queens in mature colonies of *B. mellifica,* 398, was also more than an order of magnitude higher than the other species studied (*P. emaciata* 10; *P. occidentalis* 11; *P. colobopterus* 27; *P. exigua* 20; Gastreich et al., 1993; Queller et al., 1993a; Strassmann et al., 1991, 1992). *B. mellifica* is therefore a good representative for the large-colony epiponine species that some have thought to be permanently polygynous (Jeanne, 1991; Richards, 1978; West-Eberhard, 1978).

In spite of the large differences in colony size and queen number, the genetic results from *B. mellifica* closely match those of the smaller-colony species. *B. mellifica* maintains reasonably high levels of relatedness among workers in spite of the large number of queens per colony. There are a number of factors that could cause an elevated level of relatedness between workers and the brood that they rear, including cyclical monogyny, reproductive dominance among queens, and discriminatory brood care by workers (Hughes et al., 1993, Queller, 1993). Queen dominance resulting in unequal division of reproduction among queens could raise worker relatedness levels, but it had little effect in *B. mellifica,* assuming that numbers of mature eggs in the ovaries correlates with reproduction. The same result holds for other epiponine species we previously studied (Gastreich et al., 1993; Queller et al., 1993a,c; Strassmann et al., 1991, 1992). When many queens are laying eggs, dominance must be quite extreme to cause much of an increase in the fraction of full sisters.

We did not look for within-colony kin discrimination, which is a factor that could potentially elevate relatedness among queens in this species. Conceivably, epiponine colonies might divide along genetic lines, or raise queens from only one matriline. However, the discrimination would need to be nearly perfect to get one matriline of queens out of hundreds, and our investigations of within-colony kin recognition in other species of polistine wasps have yielded entirely negative results (Queller et al., 1990; Solís et al., 1998; Strassmann et al., 1997c). Indeed, there is no uncontested evidence for within-colony kin discrimination in any species (Keller, 1997).

The main explanation for worker relatedness appears to be the same as in the other species studied: the high level of relatedness among the queens ($r = .66$ in *B. mellifica*). The most likely cause of elevated relatedness among queens is cyclical monogyny, a colony cycle in which new female reproductives are produced only when the queen number on a colony has been reduced to one; we estimate that new queens are produced on colonies with a harmonic mean of 1.2 old queens. Colonies may not remain single-queened for long, so it is not surprising that we did not include any single-queen colonies in our collection of eight colonies. Coupled with the additional finding that males are produced when colonies have more queens (as shown by the relatedness among workers on these colonies), the cycle is as predicted from worker control, with workers adopting a split sex-ratio strategy to capitalize on the three-quarters relatedness to females when they can (in single-queen colonies), and rearing males in other colonies (Queller et al., 1993c). This does not necessarily imply that workers control queen reduction, but simply that they capitalize on it properly. Under strict cyclical monogyny, we predict that queens will be full sisters ($r = .75$), workers will be cousins to each other ($r = .188$) and nieces to the average queen ($r = .375$). All of these values fall within the 95% CI for the relatedness values obtained in this study (Table 3).

Thus, it appears that cyclical monogyny and split sex ratios occur in the large colonies of *B. mellifica,* much as they do in smaller-colony species (Gastreich et al., 1993; Forsyth, 1978; Queller et al., 1988, 1993a,c; Strassmann et al., 1991, 1992; West-Eberhard, 1978). *Agelaia multipicta,* another epiponine species with large colonies (Richards, 1978) also has higher relatedness among queens (.69) than among workers (.27) (11 colonies; West-Eberhard, 1990). It therefore appears probable that cyclical monogyny is a process common to the wasp tribe Epiponini.

By generating high levels of relatedness among the new queens, worker interests appear to drive a colony cycle that fortuitously elevates all relatednesses among their progeny. We estimated the magnitude of this effect following the methods of Queller et al. (1993c). If there were no split sex ratios and new queens were produced at the same point in the colony cycle as males, we could substitute our conservative relatedness-based estimate of 19.6 queens into Equation 1 and estimate that the equilibrial queen relatedness (r and r_q) would be .05. Relatedness among their female progeny in many-queen colonies would therefore be approximately $r_q/4 = .0125$. With the actual colony cycle, which results in a $r_q = .66$, the daughter relatedness on these many-queen colonies should be .16. Thus, the worker sex-ratio preference that limits queen production to colonies of one queen and favors male production on colonies with more queens elevates worker relatednesses more than 12 times. This is the most pronounced effect of split sex ratios on relatedness in any swarm-founding wasp studied to date (Queller et al., 1993c).

The second area of collective worker interests that we explored concerns who produces the males. Workers are significantly more related to queens than they are to each other. They would therefore be more closely related to the sons of queens than to those of other workers, so they should police each other, allowing the queens to lay the male eggs. Our results indicate that queens produce most or all of the males. The males we assessed were adults, which could conceivably have moved from other colonies, but several lines of evidence argue against this (age, distribution among colonies, relatedness to colony mates). If any of the males had come from other colonies, this should have falsely shifted the likelihoods toward worker production of males because workers are more like random population members than are queens (relatedness is a measure of distinctness from the population frequencies). Thus, the possibility of intruder males could have made a finding of worker laying suspect, but our finding that queens produce most males is robust.

Thus, worker collective interests are satisfied in *B. mellifica,* with respect to both split sex ratios and the parentage of males. In the former case, collective worker interests must operate against the interests of the queens, and in the latter case, in concert with the queen's interests and against the interests of individual workers. As yet, there is no direct behavioral evidence that workers control these features in *B. mellifica,* but direct evidence for worker control exists in other social insects. There is considerable evidence that sex ratios in Hymenopteran social insects are consistent with worker interests rather than queen interests (Queller and Strassmann, 1998), and in some cases queens attempt to achieve their interests by laying more male eggs, but these are apparently eliminated by workers (Aron et al., 1994; Sundström et al., 1996).

The area of worker policing is less well studied, but some comparative evidence suggests that workers police each other's male production (Ratnieks, 1990), and honeybee workers, as predicted, remove the eggs laid by other workers (Visscher, 1996). *Brachygastra mellifica* appears to be a strong candidate for this kind of control. As in all other species of *Brachygastra* studied (Richards, 1978), there is a large class of intermediates or uninseminated females with developed ovaries. In *B. mellifica,* these are clearly workers, because they resemble other workers, but not queens, in their morphology, their genetic structure, and in their presence on the nest surface. Overall, half of the mature eggs in ovaries are found in workers rather than in queens. Because queens must lay all the female eggs from their share, the potential for worker laying of male eggs seems high. Alternatively, the intermediates may lay trophic eggs, or they may be individuals striving to become queens whose egg production is kept suppressed by other queens or workers. Resolution of this question awaits behavioral observations or genotyping of newly laid eggs.

An interesting pattern appears to be emerging from our ongoing studies of collective worker interests. As already noted, all our studies of epiponines have shown worker collective preferences to be satisfied. In contrast, our studies of *Polistes* have so far shown that collective worker interests are not met, both for the issue of queen succession in *P. annularis* (Queller et al., 1997) and for male production in *P. bellicosus* and *P. dorsalis* (Arévalo et al., 1998). One possibility is that workers cannot enforce their collective preferences in the small *Polistes* colonies, but can do so in the larger colonies of epiponines.

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